



## The complete chloroplast genome of *Callicarpa dichotoma* (Lour.) K.Koch (Lamiaceae)

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### ABSTRACT

*Callicarpa dichotoma* (Lour.) K.Koch is a shrub species with distribution from East Asia to Southeast Asia. We assembled and annotated for the first time the complete chloroplast (cp) genome of *C. dichotoma*. The cp genome of *C. dichotoma* is 154,110 bp long with the GC content of 38.09% and consists of four subregions: a large single-copy (LSC) region of 84,915 bp, a small single-copy (SSC) region of 17,783 bp and a pair of inverted repeats (IRs) of 25,706 bp each. The cp genome of *C. dichotoma* encodes a total of 114 unique genes, comprising 80 protein-coding genes, 30 tRNA genes, and four rRNA genes. Phylogenetic trees based on the coding sequences strongly support the position of *C. dichotoma* within the genus *Callicarpa*, confirming the previously reported monophyly of the genus.

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*Callicarpa*; complete chloroplast genome; Lamiaceae; medicine; phylogenetic relationships

### Introduction

The genus *Callicarpa* L. (Lamiaceae; inc. sed. Verbenaceae) is commonly known as 'beautyberry' due to their outstanding fruits, with ca. 140 species recognized worldwide (Mabberley 2017). The genus is also known for its chemical composition, such as callicarpone (Kawazu et al. 1967). The plants and their organic, plant-derived components have traditionally been used for centuries as a piscicide, pesticide, and insecticide, as well as a herbal medicine for the prevention and treatment of a wide number of health disorders in traditional pharmacology of China and other parts of Asia (Kawazu 1965; Jones and Kinghorn 2008; Tu et al. 2013; Hung et al. 2023).

*Callicarpa dichotoma* (Lour.) K.Koch 1872, or purple beautyberry, is a small, common shrub that has its natural distribution range in the temperate to subtropical zone of East Asia to Southeast Asia, from Japan to Vietnam (Yamazaki 1993; Pham 2003). *C. dichotoma* is used as an ornamental plant in America because of its colorful fruit (Bachmann 2002; Bramley 2009). However, *C. dichotoma* has recently been confirmed to be potentially invasive as it is spreading in southeastern USA (Weakley 2015; Atha et al. 2019). Although *C. dichotoma* is a common species and can cross-breed without barriers with other *Callicarpa* species, such as *C. bodinieri* H.Lév. (Xu et al. 2013), its chloroplast (cp) genome has not been elucidated. In this study, we assembled and annotated the complete cp genome of *C. dichotoma* to

better understand the position of *C. dichotoma* within the genus *Callicarpa* and for future research of the biogeography and evolution of the genus.



### Materials and methods


#### Sample collection and preservation

*Callicarpa dichotoma* (Lour.) K.Koch samples were collected from Hiroshima Prefecture, Japan (34.31N, 132.29E) (Figure 1). The voucher specimen was deposited at the Herbarium of Hiroshima University (HIRO: <http://sweetgum.nybg.org/science/ih/herbarium-details/?irn=124749>, Prof. Tomio Yamaguchi, yamatom@hiroshima-u.ac.jp) under specimen number HIRO-MY 156564.

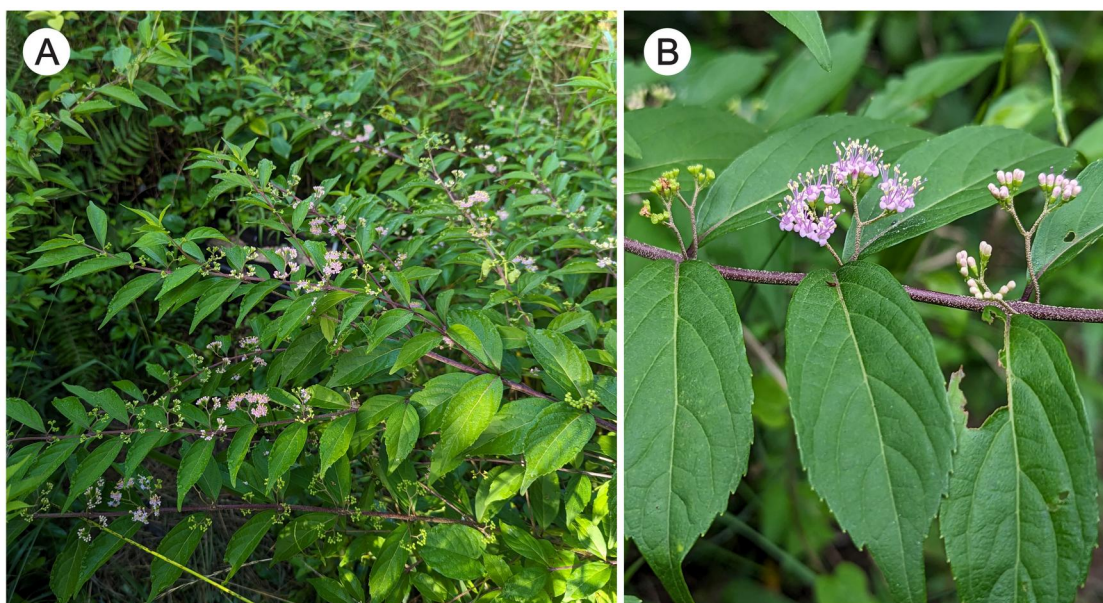
#### DNA extraction and sequencing, and cleaning raw reads

Total DNA was extracted from a frozen sample with NucleoSpin Plant II (Macherey-Nagel, Duren, Germany) following the manufacturer's instruction. Library preparation and sequencing were performed by Bioengineering Lab. Co., Ltd. (Sagamihara, Japan) using the MGI DNBSEQ-G400RS FAST platform (MGI Tech Co. Ltd., Shenzhen, China). Approximately, 77.96 M raw reads were analyzed, comprising an average fragment length of 200 bp.

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**Figure 1.** *Callicarpa dichotoma* (Lour.) K.Koch. (A) Flowering branches. (B) Close-up of flowering and fruiting branch, showing young fruits, flowers, and flower buds from left to right. Morphological features: *C. dichotoma* is a short deciduous shrub in the beautyberry genus *Callicarpa* (Lamiaceae). The branches have purplish color with stellate hairs; the leaves are simple, opposite, pubescent, ovate-oblong, acuminate at apex and cuneate at base, and serrate near the leaf tip; the cyme inflorescences are supra-axillary; the corolla is pale purple, glabrous; the stamens are exserted; and the fruits are purple.

### Assembly, annotation, and visualization

Low-quality reads (<Q30), abnormal short reads (<20 bp), and adapter sequences were trimmed using fastp ver. 0.23.2 (Chen et al. 2018). After quality control, GetOrganelle ver. 1.7.6.1 (Jin et al. 2020) was used for de novo assembly with its standard parameters and the reference data were included, and the assembled sequences were polished using Polypolish ver. 0.5.0 (Wick and Holt 2022). The polished sequences were annotated using GeSeq ver. 2.03 (Tillich et al. 2017) for protein coding and rRNA genes, and tRNAscan-SE 2.0 (Chan and Lowe 2019) for tRNA genes, and manually corrected using SnapGene Viewer ver. 7.0.2 (GSL Biotech, <https://www.snapgene.com>). To verify the accuracy of the assembly, we mapped clean reads to the assembled cp genome to assess the depth of coverage (Figure S1). A circular map was generated from the final annotated cp sequence OGDRAW ver. 1.3.1 (Greiner et al. 2019). The structures of intron-containing genes were also visualized using the CPGView online web (<http://www.1kmpg.cn/cpgview>, Liu et al. 2023; Figures S2 and S3). The cp genome sequence was submitted to the DNA Data Bank of Japan (DDBJ, <https://ddbj.nig.ac.jp/arsa/>) under the accession number LC775283.

### Phylogenetic reconstruction

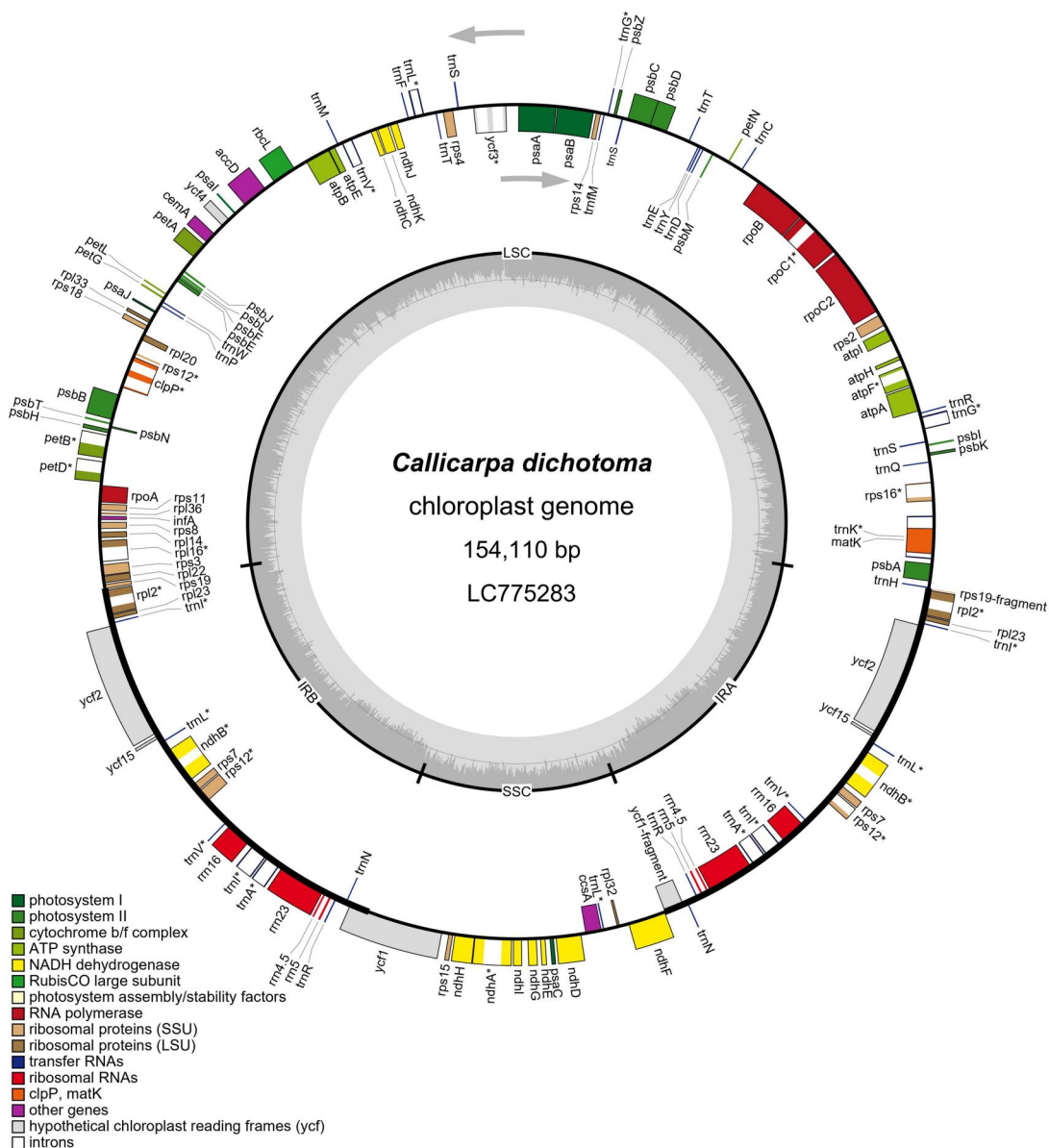
Phylogenetic analyses were conducted using protein-coding sequences (73 genes) of the cp genome. The data matrix consisted of all *Callicarpa* species with available cp genomes and closely related species in Lamiaceae (Table S1), based on the topologies demonstrated by previous studies (Li et al. 2016; Zhao et al. 2021; Liu et al. 2023). Sequences were aligned by MAFFT ver. 7.511 in the online web system (Katoh et al. 2019). After manual checking and arrangement for the alignment, start and stop codons were removed using the

sequence editor of MEGA ver. 7.0.26 (Kumar et al. 2016). Gaps were treated as missing data. ModelTest-NG ver. 0.2.0 (Darriba et al. 2020) was used to determine the substitution model for each gene based on the corrected Akaike information criterion (AICc; Sugiura 1978). The selected substitution models are listed in Table S2. RAXML-NG ver. 1.1.0 (Kozlov et al. 2019) was used for maximum-likelihood inference with a rapid bootstrap analysis of 10,000 replicates.

## Results and discussion

### Characterization of the chloroplast genome

The cp genome of *C. dichotoma* is a 154,110-bp circular DNA molecule with a typical quadripartite structure composed of a large single-copy (LSC) region of 84,915 bp, a small single-copy (SSC) region of 17,783 bp, and a pair of inverted repeats (IRs) of 25,706 bp each (Figure 2). The genome's GC content is 38.09%. It has 114 unique genes, including 80 protein-coding genes, 30 tRNA genes, and four rRNA genes. In the IR regions, 17 genes are repeated, including six protein-coding genes, seven tRNAs, and four rRNAs. The gene *rps12* is a trans-splicing gene with the first exon located in the LSC and two remaining exons in each of the IR regions. *C. dichotoma* possessed two fragments of *ycf1* and *rps19* genes (pseudogenes) located in the IRA, similar with *C. formosana* MT830861. *C. dichotoma* had a similar genome structure and gene number to *C. arborea* MT473738, *C. brevipes* MT473739, and *C. rubella* MZ520129, but differed in one protein-coding gene or one tRNA with species such as *C. integerrima* var. *chinensis* MW788028 in lacking *rpl32* gene. *C. macrophylla* MW829279 includes an additional *ycf68* gene, and *C. siog-saiensis* MW343456 lacks *trnV-UAC* (tRNA-Val). The sequence length of the cp genome obtained in this study is the third shortest among *Callicarpa* species previously reported, after



**Figure 2.** Circular map of *Callicarpa dichotoma* (Lour.) K.Koch chloroplast genome. In the chloroplast genome, the small single-copy (SSC) and large single-copy (LSC) regions are separated by inverted repeats (IRs: IRA and IRB). Genes inside the map are transcribed clockwise, and genes outside are transcribed counterclockwise. Genes with related functions are shown in the same color. Asterisks denote genes containing introns.

*C. peichieniana* (154,102 bp, MT473741) and *C. nudiflora* (154,080 bp, MK783316).

### Phylogenetic analysis of *C. dichotoma* within the genus *Callicarpa*

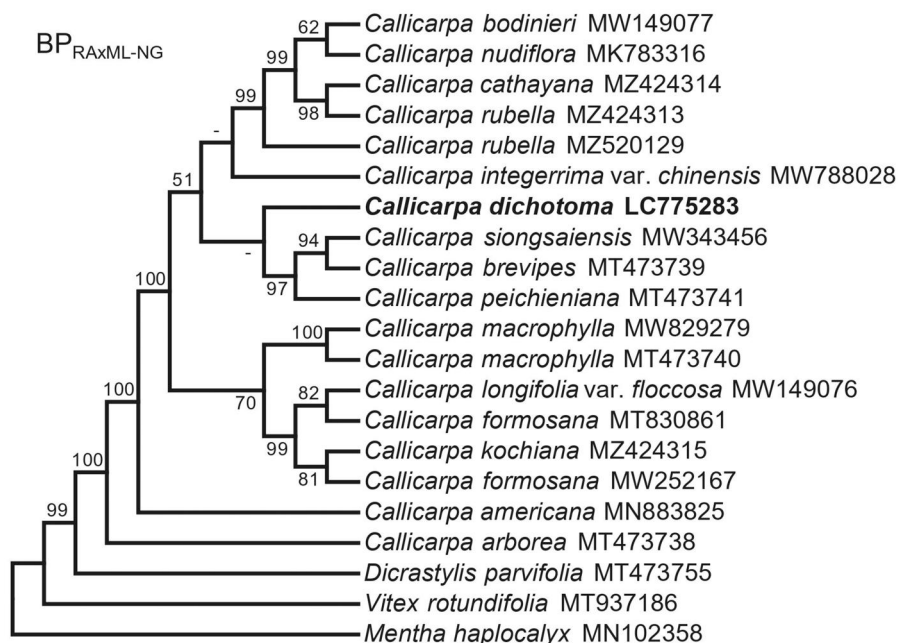
The cp genome data matrix for the phylogenetic analysis was 59,702 bp long, with 3340 variable sites (5.6%) and 589 parsimony-informative sites (17.6% of the variable sites). The phylogenetic tree fully resolved the position of *C. dichotoma* within the Asian *Callicarpa* group, thus supporting the monophyly of the genus *Callicarpa* (Figure 3). The sister relationship of *C. dichotoma* with the clade of *C. peichieniana* Chun & S.L.Chen ex H.Ma & W.B.Yu, *C. siongsaiensis* F.P.Metcalf, and *C. brevipes* (Benth.) Hance was not well resolved since the branches were very short (see the phylogram in Figure S4). The sister

relationship of *Callicarpa* (subfamily Callicarpoideae) and *Dicrastylis* (subfamily Prostantheroideae) was well supported, comparable with the results of Zhao et al. (2021).

### Conclusions

The cp genome of *Callicarpa dichotoma*, reported for the first time, had a length of 154,110 bp, and a similar cp genome structure, quadripartite structure, with that of other *Callicarpa* species. The cp genome was divided into four regions: LSC, SSC, and two IRs with the length of 84,915 bp, 17,783 bp, and 25,706 bp each, respectively. It had 114 unique, different genes, comprising 80 protein-coding genes, 30 tRNA genes, and four rRNA genes. The phylogenetic analysis supported the position of *C. dichotoma* within the Asian *Callicarpa* group. The complete cp sequence of *C. dichotoma* will





**Figure 3.** Maximum-likelihood cladogram based on 73 chloroplast protein-coding sequences for *Callicarpa* species and closely related taxa. Numbers at the branches represent the bootstrap support values of 10,000 replicates, bootstrap values under 50 were not shown. The root is arbitrarily placed on the branch leading to *Mentha haplocalyx*. The analysis involved 21 nucleotide sequences. There were a total of 59,702 positions in the final dataset. Evolutionary analyses were conducted in RAxML-NG. The following sequences were used: *Callicarpa bodinieri* MW149077 (Wang et al. 2019), *Callicarpa nudiflora* MK783316 (Wang et al. 2019), *Callicarpa cathayana* MZ424314, *Callicarpa rubella* MZ424313, *Callicarpa rubella* MZ520129 (Cai et al. 2021), *Callicarpa integerrima* var. *chinensis* MW788028 (Gu et al. 2021), *Callicarpa siongsaiensis* MW343456 (Xie et al. 2021), *Callicarpa brevipes* MT473739 (Zhao et al. 2021), *Callicarpa peichieniana* MT473741 (Zhao et al. 2021), *Callicarpa macrophylla* MW829279 (Liu et al. 2022), *Callicarpa macrophylla* MT473740 (Zhao et al. 2021), *Callicarpa longifolia* var. *floccosa* MW149076 (Wang et al. 2021), *Callicarpa formosana* MT830861 (Du et al. 2020), *Callicarpa kochiana* MZ424315, *Callicarpa formosana* MW252167, *Callicarpa americana* MN883825 (Zhao et al. 2020), *Callicarpa arborea* MT473738 (Zhao et al. 2021), *Dicrastylis parvifolia* MT473755 (Zhao et al. 2021), *Vitex rotundifolia* MT937186 (Jo et al. 2021), and *Mentha haplocalyx* MN102358 (He et al. 2020).

contribute to future studies on population genetics, biogeography, evolution, and conservation of the family.

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## Author contributions

Conceptualization: Q. C. Phan, Y. Inoue, and H. Tsubota; data curation: Q. C. Phan, R. Nagasaki, Y. Inoue, and H. Tsubota; formal analysis: H. Tsubota; funding acquisition: H. Tsubota; methodology: Q. C. Phan, R. Nagasaki, Y. Inoue, and H. Tsubota; investigation: H. Tsubota; writing – original draft preparation: Q. C. Phan; writing – review and editing: R. Nagasaki, Y. Inoue, and H. Tsubota; resources: Q. C. Phan, Y. Inoue, and H. Tsubota; all authors read and approved the final manuscript.

## Ethical approval

Research on plant organelle genome sequencing does not affect the population and does not require ethical approval. The analyzed species is widely distributed in Japan, and the material was not obtained from nature reserves.

## Disclosure statement

The authors report no potential conflict of interest.

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## Data availability statement

The genome sequence data that supports the findings of this study are openly available at NCBI (<https://www.ncbi.nlm.nih.gov/>) under the accession no. LC775283. The associated BioProject, BioSample, and SRA/DRA numbers are PRJDB15898, SAMD00611820, and DRX478534, respectively.

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